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גורמים אנטי-אלרגניים חדשים

(בעברית)
(Hebrew)

NOVEL ANTI-ALLERGENIC AGENTS

(באנגלית)
(English)

hereby apply for a patent to be granted to me in respect thereof

בקשת בזאת כי ניתן לי עליה פטנט

Application of Division	בקשת חלוקה Application for Patent-Addition	דרישת דין קידמה Priority Claim		
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חתימת המבקש
Signature of Applicant

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גורמיים אנטי-אלרגניים חדשים

NOVEL ANTI-ALLERGENIC AGENTS

NOVEL ANTI-ALLERGENIC AGENTS

FIELD OF THE INVENTION

The present invention discloses novel anti-allergenic agents, and in particular, peptidic or peptidomimetic molecules, including at least a first part which is competent for cell penetration and a second part which is able to reduce or abolish mast cell degranulation, and in particular to reduce or abolish allergy mediators such as histamine secretion from mast cells.

BACKGROUND OF THE INVENTION

Allergic diseases, including nasal allergy, asthma, urticaria and angioedema, are among the most common diseases encountered by physicians in their clinical practice. Allergy refers to certain diseases in which a wide spectrum of biologically active substances, released from activated mast cells, cause tissue inflammation and organ dysfunction. In essence, any allergic reaction may lead to tissue damage in one or more target organs (see for example Lichtenstein 1993; full references are given in the attached Appendix).

On the cellular level, mast cells are significant contributors to the allergic reaction and are packed with 500 to 1000 granules in which the mediators of the inflammatory reactions are stored. These include vasoactive mediators such as histamine, chemotactic mediators and proteolytic enzymes. In addition, following the activation of mast cells, a number of mediators are generated *de novo* and released. These include arachidonic acid metabolites such as leukotrienes and

prostaglandins and a number of multifunctional cytokines. Mast cell derived factors also recruit and activate additional inflammatory cells, such as eosinophils, neutrophils and mononuclear cells. Therefore, mast cell derived mediators possess all the requisite properties to induce the symptoms of itching, swelling, coughing and choking that are associated with an allergic reaction (Bienenstock et al., 1987).

5 These mediators are released in response to processes which occur through a number of different pathways within mast cells. Thus, therapeutic treatments for allergy and related inflammatory conditions must intervene at some point in the allergenic pathway in order to be effective.

10 Current therapies against allergy include H₁ and H₂ blockers, which block the biological activities of histamine, and leukotriene antagonists, which block the biological activities of leukotrienes. Examples include chlorpheniramine, azatidine, ketotifen, loratadine, montelukast sodium and others. However, anti-histamines or anti-leukotrienes can not counteract the inflammatory reactions effected by the 15 additional mediators released alongside histamine and leukotrienes . Therefore, these drugs can not provide reliable protection against allergy.

A better allergy treatment would block the secretory process by preventing mast cell degranulation. Drugs which are currently available for this purpose include hydrocortisone and disodium cromoglycate. However, disodium 20 cromoglycate cannot inhibit all types of histamine secretion, and is not always completely effective. Steroids, on the other hand, are effective for blocking mast cell degranulation, but have many unacceptable side effects. Therefore, therapeutic agents which could prevent mast cell degranulation without significant side effects,

and could thus prevent or significantly reduce the occurrence of clinical symptoms associated with allergy, such as neurogenic inflammation (see below for details), would be very useful for the treatment of allergy and related conditions.

Mast cell degranulation is a complex process involving at least two different pathways. Mast cells secrete their granular contents in a process of regulated exocytosis (degranulation) by two major pathways, the IgE (immunoglobulin E) dependent pathway and the IgE independent pathway. The IgE dependent pathway is invoked in response to an immunological trigger, brought about by aggregation of the high affinity receptors (F_cεRI) for IgE, which are present on the cell surface of mast cells. This response involves crosslinking of cell bound IgE antibodies by the corresponding antigens (allergens).

The IgE-independent or peptidergic pathway is invoked in response to a number of polycationic compounds, collectively known as the basic secretagogues of mast cells. These compounds include the synthetic compound 48/80, naturally occurring polyamines and positively charged peptides, such as the neurotransmitter substance P (Ennis et al., 1980; Sagi-Eisenberg 1993; Chahdi et al., 1998).

The ability of substance P to induce mast cell degranulation, together with the observed presence of mast cells clustered around nerve endings which contain substance P, implicate mast cells as the mediators of substance-P induced neurogenic inflammation (Foreman 1987a,b; Pearce et al., 1989). It is well established that in the skin and elsewhere neurogenic inflammation, through the release of neurotransmitters such as substance P, is a contributor to a variety of

diseases such as acute urticaria, psychogenic asthma, interstitial cystitis, bowel diseases, migraines, multiple sclerosis and more (Reviewed by Theoharides 1996).

In addition, this IgE independent pathway of degranulation can also be evoked by snake, bee and wasp venoms, bacterial toxins and certain drugs such as opiates.

5 Although the signal transduction pathways by which mast cell degranulation is activated are not yet fully resolved, a number of cellular events have been shown to occur after stimulation of the mast cells. These include activation of phospholipases such as PLC, PLD and PLA₂, elevation of cytosolic Ca²⁺ and activation of serine and tyrosine kinases (reviewed by Sagi-Eisenberg 1993).

10 Within these processes, however, the involvement of GTP-binding proteins (G-proteins) is well established. For example, the introduction of nonhydrolyzable analogues of GTP, such as GTP- γ -S, into ATP⁴ permeabilized mast cells, stimulates PLC activity and degranulation.

From these observations and other observations, the involvement of at least 15 two different G-proteins, one involved in PLC and Ca²⁺ activation (G_P) and one directly regulating exocytosis (G_E), has been suggested (Gomperts et al., 1991; reviewed by Sagi-Eisenberg 1993). Indeed, it was subsequently demonstrated that basic secretagogues induce histamine secretion by interacting directly with G_E, a pertussis toxin-sensitive heterotrimeric G protein, in a receptor-independent manner (Aridor et al., 1990; Aridor & Sagi-Eisenberg 1990). This G-protein was subsequently identified as G_{i3}, which appears to mediate the peptidergic pathway leading to exocytosis in mast cells. In particular, a synthetic peptide which corresponds to the C terminal sequence of G_{i3} (KNNLKECGLY) was able to

inhibit histamine release when introduced, into permeabilized mast cells (Aridor et al., 1993).

However, the cell membrane is generally impermeable to most peptides. Therefore, the use of a peptide as a therapeutic agent, directed against an 5 intracellular target, requires a special mechanism to enable the peptide to overcome the membrane permeability barrier.

One approach is based on the fusion of the selected peptide with a specific hydrophobic sequence, comprising the "h" region of a signal peptide sequence.

Examples of such hydrophobic regions are the signal sequence of the Kaposi 10 fibroblast growth factor (AAVALLPAVLLALLAP; Lin et al., 1995) and the signal sequence within human integrin β_3 (VTVLALGALAGVGVG; reviewed by Hawiger 1997). Another approach is based on fusing the active anti-allergenic peptide with a specific signal peptide sequence endowed with the membrane translocation properties of the homeodomain of Antennapedia, a *Drosophila* 15 transcription factor (RQPKIWFPNRRKPWKK; Prochiantz 1996).

Specific importation of biologically active molecules into cells by linking an importation-competent signal peptide to the molecule of interest was disclosed in U.S. Patent No. 5,807,746, although only *in vitro* studies were described, such that the signal peptide was not shown to function *in vivo*. The signal peptide causes the 20 entire complex to be imported into the cell, where theoretically the biologically active molecule could then have its effect. Although such direct importation could serve to target the therapeutic compound, and could therefore prevent or substantially reduce side effects through non-targeted system activity, the efficacy

of the complex within the cell may be limited, such that the biologically active molecule may have little or no effect once imported into the cell. The variables which may affect the efficacy of the biologically active molecule include the effect of linking the molecule to the signal peptide, which may result in an inactive complex; unpredictable effects of the entire complex within the cell; and even the inability of the entire complex to be imported into the cell, despite the presence of the signal peptide.

5 In addition, identifying a suitable biologically active molecule for treatment of allergy may also be difficult. For example, linking a non-peptide molecule, such as a known secretion-blocking compound, to a signal peptide is both difficult and may result in an unstable molecule. A peptide could be used as the secretion-blocking compound, but then such a peptide must be carefully selected and tested. Finally, the entire complex would require testing, particularly *in vivo*, since the ability to penetrate a cell in tissue culture does not necessarily predict the 10 efficacy of the complex in a human or animal subject. U.S. Patent No. 5,807,746 therefore suffers from the drawback that only *in vitro* data is disclosed, such that the effect of the signaling peptides *in vivo*, alone or as part of a complex, is not known. Thus, suitable, targeted, specific therapeutic agents for the treatment of 15 allergy are not currently available and are potentially complex and difficult to 20 develop.

There is therefore a need for, and it would be useful to have, a therapeutic agent for the treatment of allergy and related inflammatory conditions, which would block mast cell degranulation and hence the release of histamine, but which

would be specifically targeted to the degranulation pathway and which would therefore have few side effects.

SUMMARY OF THE INVENTION

5 The present invention discloses a therapeutic complex for the specific, direct and targeted treatment of allergies and related inflammatory conditions, which includes at least a first moiety which is competent for the importation of the complex into mast cells, and a second moiety which is able to block or significantly reduce mast cell degranulation and hence the release of histamine. For 10 example, the first moiety could include a signal peptide which is competent for the importation of the complex into mast cells, while the second moiety could include a biologically active molecule, such as a peptide which is able to block the G protein-mediated contribution to the mast cell degranulation process.

According to the present invention, there is provided an anti-allergic 15 complex, comprising a molecule having at least a first moiety competent for importation of the molecule into mast cells, and a second moiety for having an anti-allergic effect within the mast cells, the first moiety being joined to the second moiety through a linker.

Preferably, the second moiety has the anti-allergic effect by at least 20 significantly reducing degranulation of the mast cells. More preferably, the second moiety is selected from the group consisting of a peptide, a polypeptide, a protein, a nucleic acid, a carbohydrate, a lipid, and a glycolipid. Most preferably, the second moiety is a peptide. Also most preferably, the first moiety is a peptide.

According to a preferred embodiment of the present invention, the linker is a covalent bond. Preferably, the covalent bond is a peptide bond. More preferably, the molecule is a peptide taken from the C terminal sequence of Gαi₃. Most preferably, the peptide has an amino acid sequence

5 AAVALLPAVLLALLAPKNNLKECGLY.

According to another particularly preferred embodiment of the present invention, the molecule is a peptide having an amino acid sequence
AAVALLPAVLLALLAPKENLKDCGLF.

According to another embodiment of the present invention, there is provided
10 a composition for treating an allergenic condition in a subject, comprising a pharmaceutically effective amount of a therapeutic agent for administering to the subject, the therapeutic agent comprising a molecule having at least a first moiety competent for importation of the molecule into mast cells, and a second moiety for having an anti-allergic effect within the mast cells, the first moiety being joined to
15 the second moiety through a linker.

Preferably, the allergenic condition is selected from the group consisting of nasal allergy, an allergic reaction in an eye of the subject, an allergic reactions in the skin of the subject, acute urticaria, psoriasis, psychogenic or allergic asthma, interstitial cystitis, bowel diseases, migraines, and multiple sclerosis. More
20 preferably, the therapeutic agent is administered by topical administration. Most preferably, the topical administration is to the skin of the subject.

According to a preferred embodiment of the present invention, the therapeutic agent is administered by inhalation. Alternatively and preferably, the

therapeutic agent is administered intranasally.

According to another preferred embodiment of the present invention, the molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY. Alternatively and preferably, the 5 molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKENLKDCGLF. More preferably, the therapeutic agent further comprises a second molecule, the second molecule being a peptide having an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.

According to still another embodiment of the present invention, there is 10 provided a method for treating an allergic condition in a subject, comprising the step of administering a pharmaceutically effective amount of a therapeutic agent to the subject, the therapeutic agent comprising a molecule having at least a first moiety competent for importation of the molecule into mast cells, and a second moiety for having an anti-allergic effect within the mast cells, the first moiety 15 being joined to the second moiety through a linker.

According to yet another embodiment of the present invention, there is provided a method for promoting importation of a molecule into a cell of a subject *in vivo*, the method comprising the steps of: (a) attaching a leader sequence to the molecule, the leader sequence being a peptide having an amino acid sequence 20 AAVALLPAVLLALLAP, to form a complex; (b) administering the complex to the subject; and (c) importing the complex into the cell through the leader sequence, such that the molecule is imported into the cell.

thereof, which are capable of exerting an effect in a biological system. Hereinafter, the term "fragment" refers to a portion of a molecule or a complex thereof, in which the portion includes substantially less than the entirety of the molecule or the complex thereof.

5 Hereinafter, the term "amino acid" refers to both natural and synthetic molecules which are capable of forming a peptide bond with another such molecule. Hereinafter, the term "natural amino acid" refers to all naturally occurring amino acids, including both regular and non-regular natural amino acids. Hereinafter, the term "regular natural amino acid" refers to those alpha amino acids
10 which are normally used as components of a protein. Hereinafter, the term "non-regular natural amino acid" refers to naturally occurring amino acids, produced by mammalian or non-mammalian eukaryotes, or by prokaryotes, which are not usually used as a component of a protein by eukaryotes or prokaryotes.
Hereinafter, the term "synthetic amino acid" refers to all molecules which are
15 artificially produced and which do not occur naturally in eukaryotes or prokaryotes, but which fulfill the required characteristics of an amino acid as defined above. Hereinafter, the term "peptide" includes both a chain of a sequence of amino acids, whether natural, synthetic or recombinant. Hereinafter, the term "peptidomimetic" includes both peptide analogues and mimetics having
20 substantially similar or identical functionality thereof, including analogues having synthetic and natural amino acids, wherein the peptide bonds may be replaced by other covalent linkages.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described; by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 is a graph of the effect of different peptides on histamine secretion;

5 FIG. 2 is a graph of the dose effect of compound 48/80 induced histamine release from intact mast cells;

FIG. 3 is a graph of the inhibitory effect of peptides 1 and 4 on histamine secretion;

10 FIG. 4 is a graph of the inhibitory effect of peptides 2 and 5 on histamine secretion;

FIG. 5 is a graph of the inhibitory effect of peptide 2 on histamine secretion; and

15 FIG. 6 shows the inhibitory effect of peptide 2 on substance P induced histamine secretion.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention discloses a therapeutic complex for the specific, direct and targeted treatment of allergies and related inflammatory conditions, which comprises molecules having at least a first moiety which is competent for the 20 importation of the complex into mast cells, and a second moiety which is able to block or significantly reduce mast cell degranulation and hence the release of histamine. The first moiety is connected to the second moiety through a linker.

The first moiety is a molecule, preferably a peptide, and more preferably a

signal peptide. A signal peptide is a peptide which is capable of penetrating through the cell membrane, to permit the exportation and/or importation of proteins or peptides. As used herein, suitable signal peptides are those which are competent for the importation of proteins, peptides or other molecules into the cell. Such 5 signal peptides generally feature approximately 10-50 amino acids, of which a majority are typically hydrophobic, such that these peptides have a hydrophobic, lipid-soluble portion. Preferably, signal peptides are also selected according to the type of cell into which the complex is to be imported, such that signal peptides produced by a particular cell type, or which are derived from peptides and/or 10 proteins produced by that cell type, can be used to import the complex into cells of that type. Examples of such signal peptides are described above and are also disclosed in U.S. Patent No. 5,807,746, incorporated by reference as if fully set forth herein for the teachings regarding signal peptides.

The second moiety is a molecule which has an anti-allergic effect, 15 preferably by preventing mast cell degranulation, and hence the release of histamine from these mast cells. The molecule is preferably a peptide, and more preferably a peptide derived from the C terminal sequence of Gαi₃, which appears to mediate the peptidergic pathway leading to exocytosis in mast cells.

Alternatively, the second moiety is selected from the group consisting of a 20 polypeptide, a protein, a nucleic acid, a carbohydrate, a lipid, and a glycolipid.

The linker which connects the first moiety to the second moiety is preferably a covalent bond, and more preferably a peptide bond if at least one of the first and second moieties is a peptide. A non-peptidic covalent bond could be

formed by methods which are known in the art, such as for example cross-linking the first and the second moieties with a cross-linking reagent, such as glutaraldehyde for example.

The present invention also discloses methods for treating allergies.

5 Hereinafter, the term "treatment" includes both the prevention of the allergic condition, as well as the substantial reduction or elimination of allergic symptoms. Allergenic conditions for which the therapeutic agents of the present invention are useful include, but are not limited to, nasal allergy, irritation or allergic reactions in the eyes, allergic reactions in the skin including any type of allergen-induced rash 10 or other skin irritation or inflammation, acute urticaria, psoriasis, psychogenic or allergic asthma, interstitial cystitis, bowel diseases, migraines, and multiple sclerosis.

Such treatment may be performed topically, for example for skin allergies and allergic reactions, including but not limited to, contact dermatitis in reaction to 15 skin contact with an allergen; reactions to insect bites and stings; and skin reactions to systemic allergens, such as hives appearing after a food substance has been ingested by the subject. Alternatively and/or additionally, such treatment may be performed by systemic administration of the therapeutic complex. A preferred route of administration is oral, but alternative routes of administration include, but 20 are not limited to, intranasal, intraocular, sub-cutaneous and parenteral administration. Other routes of administration, and suitable pharmaceutical formulations thereof, are described in greater detail below.

As noted previously, in the most preferred embodiment of the present

invention, the first and the second moieties are both peptides which are joined with a peptide bond. In the present invention, novel peptides, designated as peptides 1-6, were designed and synthesized to include distinct importation competent signal peptides as a first moiety at the N-terminus (underlined), and to include peptides from the C-terminal sequences of G_{αi3} or G_{αt} at the C-terminus as a second moiety. The second moiety is taken from G_{αi3} for peptides 1-3, and is taken from G_{αt} for peptides 4-6. These peptides are listed as follows:

1. VTVLALGALAGVGVGKNNLKECGLY
2. AAVALLPAVLLALLAPKNNLKECGLY
- 10 3. RQPKIWFPNRRKPWKKKNNLKECGLY
4. VTVLALGALAGVGVGKENLKDCGLF
5. AAVALLPAVLLALLAPKE NLKDCGLF
6. RQPKIWFPNRRKPWKKKENLKDCGLF

These peptides were then examined *in-vitro* for their ability to block compound 48/80 induced histamine secretion from purified rat peritoneal mast cells (Aridor *et al.*, 1993). Peptides 2 and 5 were demonstrated as blockers of compound 48/80 induced histamine secretion from mast cells in a dose response effect. Peptide 2 was more potent than peptide 5, however when applied together both peptides showed a synergistic effect for inhibiting histamine release. Peptides 20 1,3,4, and 6 showed no inhibition.

Peptides 2 and 5, and their homologues or structurally similar related compounds, could therefore be useful for non-immunological (IgE-independent) allergies, in which mast cell degranulation is mediated through the

IgE-independent pathway from which the second moiety of the above peptides was taken. Examples of such allergies include but are not limited to neurogenic inflammation in the skin and elsewhere, including but not limited to, acute urticaria, psoriasis, psychogenic asthma, interstitial cystitis, bowel diseases, 5 migraines, and multiple sclerosis. In addition, the leader sequence (first moiety) of peptides 2 and 5 could also be useful for the importation of a molecule into the cell of a subject *in vivo*, as such an effect had not been previously demonstrated in the background art.

10

EXAMPLE 1
TESTING OF PEPTIDES 1-6 IN VITRO

15

Peptides 1-6 of the present invention, as described above, were tested *in vitro* for their ability to block histamine secretion from mast cells. Rat peritoneal mast cells were chosen as the experimental model, since it was previously shown that both rat peritoneal and human skin mast cells release histamine in response to substance P by an IgE-independent mechanism (Devillier et al., 1986; Foreman 1987a,b; Columbo et al., 1996). It was also demonstrated that the same peptidergic pathway is involved in both rat peritoneal and human cutaneous mast cells (Mousli et al., 1994; Emadi-Khiav et al., 1995).

20

Compound 48/80 was chosen as the allergen since it is one of the polycationic compounds, collectively known as the basic secretagogues of mast cells. Compound 48/80 has been shown to induce degranulation of human mast cells. In particular, it is very active on skin mast cells. Compound 48/80 has been used as a diagnostic agent *in vivo* to assess the release ability of human mast cells,

to determine the effectiveness of drugs against chronic urticaria and to study itch and flare responses in atopic dermatitis (Kivity et al., 1988; Goldberg et al., 1991). Therefore inhibition of compound 48/80 induced histamine release is applicable and relevant to prevention of allergy induced by other basic secretagogues such as 5 substance P, snake, bee and wasp venoms, bacterial toxins and certain drugs such as opiates.

The ability of each of peptides 1-6 to inhibit mast cell degranulation, when induced by compound 48/80, was then tested. The experimental method was as follows.

10

MATERIALS AND METHODS

Peptide synthesis

Peptides were synthesized by IMI (Institute for Research and Development Ltd., Haifa, Israel). Peptides were synthesized by the solid phase methodology and supplied at > 95% 15 purity. The correct composition and purity of the peptides were verified by HPLC, mass spectrometry and amino acid analysis. Peptides stock solutions (5 mg/ml in 10% dimethylsulfoxide (DMSO) in H₂O) were kept at 20 °C.

Isolation and purification of mast cells

20 Mast cells from the peritoneal cavity of C.R rats were isolated in Tyrode buffer (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 0.4 mM NaH₂PO₄, 20 mM Hepes, 1.0 mM CaCl₂, 5.6 mM glucose, 1 mg/ml BSA, pH 7.2) and purified over a Ficoll gradient. A suspension of washed peritoneal cells was placed over a cushion

of 30% Ficoll 400 (Pharmacia Biotech.) in buffered Saline containing 0.1% BSA, and centrifuged at 150xg for 15 min. The purity of mast cells recovered from the bottom of the tube was > 90%, as assessed by toluidine blue staining.

5

Triggering histamine secretion from intact cells

Purified mast cells (duplicated of 10^5 cells/0.5ml) were incubated in Tyrode buffer with buffer or with desired concentrations of the indicated peptide for 2 h. at 37°C. Histamine secretion was subsequently stimulated by the indicated concentration of compound 48/80 (Sigma) dissolved in Tyrode buffer. Incubation 10 with compound 48/80 was carried out for 20 min. at 37°C. The reaction was terminated by placing the tubes in ice. The cells were sedimented by centrifugation at 150xg for 5 min. and the supernatants were collected. The amount of histamine release was determined as previously described (Aridor et al., 1990). Briefly, cell pellets were lysed using 0.1N NaOH and the volume of each sample was adjusted 15 to 0.5 ml by H₂O. Histamine content was assayed using the o-phtalaldehyde (OPT) fluorimetric method (Shore et al., 1959). Aliquots of 0.4 ml from the supernatants and cell lysates were incubated with 1.6 ml H₂O, 0.4 ml 1N NaOH and 0.1 ml of 10 mg/ml OPT in methanol, for 4 min. at room temperature. The reaction was terminated by the addition of 0.2 ml 3N HCl. Samples were centrifuged at 150xg 20 for 5 min. and 0.2 ml samples were transferred to a 96 well plate. The histamine spectrofluorometric assay was run in microplates using a microplates reader (FL-600, Biotek instruments Winooski, VT, USA). Samples were excited by light at 340nm and read at 440nm. Histamine release was calculated as the percentage of

total histamine content (supernatant / pellet + supernatant) in each sample. Each data point represents the average of duplicate measurements. The spontaneously released histamine was subtracted. Statistical analysis and plotting were done with Excel® (Microsoft Ltd., Washington, USA).

5

Induction of Histamine Secretion by Substance P

The induction of histamine secretion was performed by 50 µM of the physiological basic secretagogue substance P, and thus mimics histamine release in vivo. In these experiments, mast cells were incubated with increasing concentrations of peptide 2 for 2 h. at 37 °C. Following the two hour incubation period, histamine secretion was stimulated by 5 µl of substance-P at a final concentration of 50 µM. Histamine release was determined as previously described, and is presented as percentage of the maximal response, which corresponds to 22% of the total cellular histamine content.

15

RESULTS

As demonstrated in Figure 1, peptides 1 and 4 (which both include the leader motif of the signal sequence within human integrin β_3 linked to the C-terminal sequences of Gai $_3$ or Gat, respectively) exerted hardly any stimulatory effect on histamine secretion at a concentration range of up to 400 µg/ml of the peptide (Figure 1A). Similar results were obtained with peptides 2 and 5 (which both include the leader motif of the signal sequence of the Kaposi fibroblast growth factor linked to the C-terminal sequences of Gai $_3$ or Gat, respectively) at a

concentration range of up to 600 $\mu\text{g}/\text{ml}$ of the peptide (Figure 1B). In contrast, peptides 3 and 6 (which both include the leader motif of the homeodomain of a *Drosophila* transcription factor linked to the C-terminal sequences of $\text{G}\alpha_i$ or $\text{G}\alpha_t$, respectively) induced histamine secretion from mast cells in a concentration dependent manner (Figure 1C). These results suggest that peptides containing the h region of a signal peptide sequence of either the signal sequence of the Kaposi fibroblast growth factor or the signal sequence within human integrin β_3 , can serve as potential inhibitors of mast cells exocytosis, as they do not exert side effects of effecting histamine secretion. In contrast, peptides including the leader motif of the homeodomain of the *Drosophila* transcription factor induce side effects of histamine secretion, and therefore can not serve as potential inhibitors of mast cells exocytosis.

In order to investigate the ability of peptides 1, 2, 4 and 5, which did not exert side effects on mast cells *in vitro*, to block allergen-induced exocytosis, these peptides were examined for their ability to block compound 48/80 induced histamine secretion from intact mast cells *in vitro*.

First, the effect of compound 48/80 on exocytosis from intact mast cells was determined. A calibration curve is demonstrated in Figure 2, displaying a dose response of histamine release induced by compound 48/80. Half-maximal release was obtained at 0.1 $\mu\text{g}/\text{ml}$ while maximal release was obtained at 1-10 $\mu\text{g}/\text{ml}$. Accordingly, in order to analyze the potential of each peptide to block histamine secretion, histamine secretion was induced with 5 $\mu\text{g}/\text{ml}$ compound 48/80, a concentration that induces a maximal histamine release, while the cells were

incubated in the presence of increasing concentrations of each peptide.

Peptides 1 and 4 (which include the leader motif of the signal sequence within human integrin β_3 and the C-terminal sequences of $G\alpha_i$ or $G\alpha_t$, respectively) exerted hardly any blocking effect on histamine secretion that was induced by compound 48/80, at a concentration range of up to 400 $\mu\text{g}/\text{ml}$ of the peptide (Figure 3). Applying both peptides simultaneously in a concentration range of even up to 600 $\mu\text{g}/\text{ml}$ did not reveal any synergistic effect. Thus, peptides 1 or 4, which include the leader motif of the signal sequence within human integrin β_3 , although exerting no side effects on mast cells in our *in vitro* system, do not block histamine secretion, thus can not serve as a potential inhibitors of mast cells exocytosis.

On the other hand, peptides 2 and 5 (which both include the leader motif of the signal sequence of the Kaposi fibroblast growth factor linked to the C-terminal sequences of $G\alpha_i$ or $G\alpha_t$, respectively) exerted inhibition of histamine secretion that was induced by compound 48/80 (Figure 4). Peptide 5 demonstrated very moderate inhibition of up to 16% of the maximal response at a concentration of 800 $\mu\text{g}/\text{ml}$. Peptide 2 was more potent and inhibited by 25% the maximal response, at a concentration of 600 $\mu\text{g}/\text{ml}$. Applying both peptides simultaneously revealed a synergistic effect causing more extensive inhibition of histamine secretion at a concentration dependent manner (Inhibition of 25 %, 38% and 45% of the maximal response at concentrations of 400, 600 and 800 $\mu\text{g}/\text{ml}$, respectively). Thus, peptides 2 and 5 can serve as potential inhibitors of mast cell exocytosis, while exerting no side effects in this experimental system.

In order to examine further the ability of peptide 2 to serve as a blocker of histamine release, induction of histamine secretion was performed by 0.1 μ g/ml compound 48/80, a concentration that was demonstrated previously to cause half-maximal release of histamine (Figure 2), and thus mimics histamine release *in vivo* occurring following exposure to substance-P or other physiological basic secretagogues. As shown in Figure 5, the results demonstrate that peptide 2 exerted inhibition of histamine secretion induced by 0.1 μ g/ml of compound 48/80. This inhibition was dose dependent with maximal inhibition of 84% achieved at a concentration of 600 μ g/ml peptide.

Figure 6 shows that peptide 2 also exerted inhibition of histamine secretion induced by substance P. This inhibition was dose dependent with maximal inhibition of 67% achieved at a concentration of 400 μ g/ml peptide. Thus, peptide 2 has the potential to fully block histamine release from mast cells induced by physiological concentrations of basic secretagogues. This peptide may thus provide a unique and effective means to block mast cell exocytosis that leads to the allergic reaction.

EXAMPLE 2
TESTING OF THE EFFECTS OF THE TREATMENT
OF THE PRESENT INVENTION IN VIVO

The ability of peptide 2 to block the release of histamine secretion *in vivo* was tested on the skin of rats by using compound 48/80 as the allergen. Peptide 2 was shown to also effectively block the allergic response by preventing the release of histamine from mast cells *in vivo*. The experimental method was as follows.

MATERIALS AND METHODS

The hair of the abdominal area of C.R rats was carefully removed with an electric clipper and a depilatory cream. In each animal the abdominal area was 5 divided to six equal zones that were marked by pen. Each zone was either subjected to peptide treatment or served as a control. In the first set of experiments, the peptide was topically applied as follows: 36 μ l of peptide solution at the indicated concentration (dissolved in 72% DMSO in saline) was applied on desired abdominal area. In the second set of experiments, the peptide was injected 10 intradermally as follows: 20 μ l of peptide solution at different concentrations (dissolved in 10% DMSO in saline) was injected intradermally to an indicated abdominal area using a 27-gauge sterile needle.

Skin tests were performed 0.5, 1 or 2 hours following application of the peptide. Skin tests were performed by injecting intradermally 20 μ l of the allergen 15 (0.1 mg/ml compound 48/80 dissolved in saline) or saline alone, into the center of each marked area on the abdominal skin using a 27-gauge sterile needle. The allergic response was monitored by outlining with a marker the wheals which developed in response to allergen or saline treatment.

To quantitate the skin test results, the marker signs were transferred onto 20 paper with scotch tape. The areas of the wheals were outlined and calculated by a computerized planimeter (Hewlett-Packard), as previously described (Sussman et al., 1982).

RESULTS

The area of the wheals which developed in response to topical application of peptide 2 followed by compound 48/80 or saline injection is given in Table 1, with the net wheal area given in Table 2. These results demonstrate that the size of the wheals which developed after saline injection range between 72-93 mm² while the size of the wheals which developed after the injection of compound 48/80 range between 114-134 mm², demonstrating a significant allergic response induced by intradermal injection of compound 48/80 as compared to saline. The wheals which developed following the application of peptide 2 and saline injection, without compound 48/80, were slightly smaller than the wheals developed following no peptide application (Table 2A). These results demonstrate that topical application of peptide 2 by itself exerts no stimulatory effect on the cutaneous allergic reactions.

Comparing the net allergic reaction, which is the wheal area induced by saline injection subtracted from the wheal area induced by the injection of compound 48/80 injection, reveal that topical application of 350 micrograms of peptide 2 reduced compound 48/80-induced allergic reaction from a net wheal area of 41-42 mm² to a net wheal area of 9-12 mm² (Table 2B).

These results further reinforce the *in vitro* results, demonstrating that peptide 2 has the potential to also block allergic reactions *in vivo*, such as the cutaneous allergic reactions. The area of the wheals which developed in response to intradermal injection of peptide 2 followed by compound 48/80 or saline injection is given in Table 3, with the net wheal area given in Table 4. The wheals,

which developed following the injection of peptide 2 and saline, without compound 48/80, were similar to the wheals developed following no peptide application (Table 3, 4A). These results demonstrate that intradermal injection of peptide 2 by itself exerts hardly any stimulatory effect on the cutaneous allergic reactions.

Comparing the net allergic reaction, which is the wheal area induced by saline injection subtracted from the wheal area induced by the injection of compound 48/80, reveal that intradermal injection of 20 µg of peptide 2 already reduced compound 48/80-induced allergic reaction (Table 4B), while a higher dose of 200 µg of the peptide, increased the inhibition effect (Table 4B).

Table 1: Wheal areas (mm²) in response to topical application of peptide 2 followed by injection of saline or compound 48/80.

Peptide application Skin test	Peptide concentration		
	0	1 mg/ml	10 mg/ml
Saline injection (control)	93.52 ^a 72.39 ^b	84.64 ^a 66.91 ^b	85.24 ^a 66.40 ^b
compound 48/80 injection	134.50 ^a 114.80 ^b	126.00 ^a 106.70 ^b	94.46 ^a 85.48 ^b

15

^a Animal a - Skin tests were performed 1 hour after application of the peptide.

^b Animal b - Skin tests were performed 2 hours after application of the peptide.

Table 2: Net wheal areas (mm²) in response to topical application of peptide 2

A: Effect of Peptide (alone)

Peptide application Animal	Peptide concentration	
	1 mg/ml	10 mg/ml
A*	-8.8	-8.3
B**	-5.5	-6.0

5

B: Effect of Peptide on 48/80 induced weal response

Peptide application Animal	Peptide concentration		
	0	1 mg/ml	10 mg/ml
A*	41.0	41.4	9.2
B**	42.4	39.8	19.1

* Animal A - Skin tests were performed 1 hour after application of the peptide.

10 **Animal B - Skin tests were performed 1 hour after application of the peptide.

Table 3: Wheal areas (mm²) in response to intradermal injection of peptide 2 followed by injection of saline or compound 48/80.

Peptide concentration Skin test	0	1 mg/ml	10 mg/ml
Saline injection (control)	62.49 ^a	65.78 ^a	60.81 ^a
	112.2 ^b	119.2 ^b	102.3 ^b
	60.81 ^c	77.31 ^c	57.81 ^c
compound 48/80 injection	175.3 ^a	141.5 ^a	82.06 ^a
	175.3 ^b	120.8 ^b	107.6 ^b
	117.6 ^c	118.5 ^c	72.77 ^c

5 ^a Animal a - Skin tests were performed 0.5 hour after injection of the peptide.

^b Animal b - Skin tests were performed 1 hour after injection of the peptide.

^c Animal c - Skin tests were performed 2 hours after injection of the peptide.

Table 4: Net wheal areas (mm^2) in response to intradermal injection of peptide 2

A: Effect of Peptide (alone)

Peptide concentration Animal	1 mg/ml	10 mg/ml
A*	3.29	-1.68
B**	7.0	-9.9
C***	16.5	-3.0

5

B: Effect of Peptide on compound 48/80 induced weal response

Peptide concentration Animal	0	1 mg/ml	10 mg/ml
A*	112.8	75.7	21.3
B**	63.1	1.6	5.3
C***	56.8	41.2	15

* Animal A - Skin tests were performed 0.5 hour after injection of the peptide.

10 **Animal B - Skin tests were performed 1 hour after injection of the peptide.

***Animal C - Skin tests were performed 2 hours after injection of the peptide.

EXAMPLE 3
METHODS AND COMPOSITIONS FOR ADMINISTRATION

The peptides of the present invention, and their homologues or related compounds, hereinafter referred to as the "therapeutic agents of the present invention", can be administered to a subject by various ways, which are well known in the art. Hereinafter, the term "therapeutic agent" includes a peptide as previously defined, in particular peptide 2 and/or homologues, analogues or mimetics thereof, or any biologically active substance having a substantially similar effect as previously defined.

Hereinafter, the term "subject" refers to the human or lower animal to whom the therapeutic agent is administered. For example, administration may be done topically (including ophthalmically, vaginally, rectally, intranasally and by inhalation), orally, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, or intramuscular injection.

Formulations for topical administration may include but are not limited to lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions which may also contain buffers, diluents and other suitable

additives.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the subject to the therapeutic agent. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

EXAMPLE 4
METHOD OF TREATMENT OF ALLERGENIC CONDITIONS

As noted above, the therapeutic agents of the present invention have been shown to be effective inhibitors of the allergenic process by blocking mast cell degranulation, thereby preventing and/or alleviating an allergenic condition. The following example is an illustration only of a method of treating an allergenic condition with the therapeutic agent of the present invention, and is not intended to be limiting.

The method includes the step of administering a therapeutic agent, in a pharmaceutically acceptable carrier as described in Example 3 above, to a subject to be treated. The therapeutic agent is administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as the absence of a symptom of the allergenic condition in the subject, or the prevention of the appearance of such a symptom in the subject.

Allergenic conditions for which the therapeutic agents of the present invention are useful include, but are not limited to, nasal allergy, irritation or allergic reactions in the eyes, allergic reactions in the skin including any type of allergen-induced rash or other skin irritation or inflammation, acute urticaria,

psoriasis, psychogenic or allergic asthma, interstitial cystitis, bowel diseases, migraines, and auto-immune diseases such as multiple sclerosis.

EXAMPLE 5

5 METHODS FOR MANUFACTURING
 THE THERAPEUTIC COMPLEX OF THE PRESENT INVENTION

The therapeutic complex of the present invention can be manufactured in various ways. For example, if the therapeutic complex includes a peptide for at 10 least one the first moiety and the second moiety, or if the entire therapeutic complex is a peptide, then such a peptide could be manufactured by peptide synthetic methods which are well known in the art.

Alternatively, such a peptide could be produced by linking the signal sequence and the biologically active moiety through laboratory techniques for 15 molecular biology which are well known in the art.

By way of illustration , as a non-limiting example, a recombinant fusion protein could be prepared which would feature the peptide permeabilization sequence in the N-terminus and the C-terminal moiety of G α t or G α i₃, preferably including the last 10 amino acids, for production in bacteria. For this purpose, 20 DNA sequences coding for the desired peptides are amplified by PCR and purified. After sequence verification, these DNA sequences are ligated and cloned in an appropriate vector. The resulting recombinant plasmid is expressed in E. coli and the recombinant proteins purified from bacterial extracts.

According to another preferred embodiment of the present invention, a 25 peptide could optionally be modified. For example, the N-terminus of the peptide

could be modified by succinylation, addition of a sugar residue, or addition of stearic or palmitic acid. In addition, certain amino acids of the peptide could also be modified. For example, if the peptide includes a cysteine at amino acid 23, this cysteine could be replaced by another amino acid, including but not limited to, 5 amino butyric acid, serine or other such amino acids. As another example, if the peptide includes a lysine at amino acid 17, this residue could be replaced by another amino acid, such as a neutral amino acid, or two amino acids such as a pair of glutamic acid residues. As yet another example, if the peptide includes a proline at amino acid 16, this residue could be replaced by another amino acid, such as a 10 neutral amino acid, or two amino acids such as a pair of glutamic acid residues.

Thus, the peptide could optionally be modified in order to enhance penetration into the cell or to enhance the pharmaceutical activity, for example.

It will be appreciated that the above descriptions are intended only to serve 15 as examples, and that many other embodiments are possible within the spirit and the scope of the present invention.

WHAT IS CLAIMED IS:

1. An anti-allergic complex, comprising a molecule having at least a first moiety competent for importation of said molecule into mast cells, and a second moiety for having an anti-allergic effect within said mast cells, said first moiety being joined to said second moiety through a linker.
2. The complex of claim 1, wherein said second moiety has said anti-allergic effect by at least significantly reducing degranulation of said mast cells.
3. The complex of claim 2, wherein said second moiety is selected from the group consisting of a peptide, a polypeptide, a protein, a nucleic acid, a carbohydrate, a lipid, and a glycolipid.
4. The complex of claim 3, wherein said second moiety is a peptide.
5. The complex of claim 4, wherein said first moiety is a peptide.
6. The complex of claim 5, wherein said linker is a covalent bond.
7. The complex of claim 6, wherein said covalent bond is a peptide bond.

8. The complex of claim 7, wherein said second moiety is a peptide taken from the C terminal sequence of $\text{G}\alpha_1$.

9. The complex of claim 8, wherein said peptide has an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.

10. The complex of claim 7, wherein said second moiety is a peptide taken from the C terminal sequence of $\text{G}\alpha_t$.

11. The complex of claim 10, wherein said molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKENLKDCGLF.

12. A composition for treating an allergenic condition in a subject, comprising a pharmaceutically effective amount of a therapeutic agent for administering to the subject, said therapeutic agent comprising a molecule having at least a first moiety competent for importation of said molecule into mast cells, and a second moiety for having an anti-allergic effect within said mast cells, said first moiety being joined to said second moiety through a linker.

13. The composition of claim 12, wherein the allergenic condition is selected from the group consisting of nasal allergy, an allergic reaction in an eye of the subject, an allergic reactions in the skin of the subject, acute urticaria, psoriasis, psychogenic or allergic asthma, interstitial cystitis, bowel diseases, migraines, and

multiple sclerosis.

14. The composition of claim 13, wherein said therapeutic agent is administered by topical administration.

15. The composition of claim 14, wherein said topical administration is to the skin of the subject.

16. The composition of claim 13, wherein said therapeutic agent is administered by inhalation.

17. The composition of claim 13, wherein said therapeutic agent is administered intranasally.

18. The composition of claim 13, wherein said second moiety has said anti-allergic effect by at least significantly reducing degranulation of said mast cells.

19. The composition of claim 18, wherein said second moiety is selected from the group consisting of a peptide, a polypeptide, a protein, a nucleic acid, a carbohydrate, a lipid, and a glycolipid.

20. The composition of claim 19, wherein said second moiety is a

peptide.

21. The composition of claim 20, wherein said first moiety is a peptide.
22. The composition of claim 21, wherein said linker is a covalent bond.
23. The composition of claim 22, wherein said covalent bond is a peptide bond.
24. The composition of claim 23, wherein said second moiety is a peptide taken from the C terminal sequence of G α i₃.
26. The composition of claim 25, wherein said molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.
27. The composition of claim 23, wherein said second moiety is a peptide taken from the C terminal sequence of G α t.
28. The composition of claim 27, wherein said molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKENEKDCGLF.
29. The composition of claim 28, wherein said therapeutic agent further comprises a second molecule, said second molecule being a peptide having an

amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.

30. A method for treating an allergenic condition in a subject, comprising the step of administering a pharmaceutically effective amount of a therapeutic agent to the subject, said therapeutic agent comprising a molecule having at least a first moiety competent for importation of said molecule into mast cells, and a second moiety for having an anti-allergic effect within said mast cells, said first moiety being joined to said second moiety through a linker.

31. The method of claim 30, wherein the allergenic condition is selected from the group consisting of nasal allergy, an allergic reaction in an eye of the subject, an allergic reactions in the skin of the subject, acute urticaria, psoriasis, psychogenic or allergic asthma, interstitial cystitis, bowel diseases, migraines, and multiple sclerosis.

32. The method of claim 31, wherein the step of administering said therapeutic agent is performed by topical administration.

33. The method of claim 32, wherein said topical administration is to the skin of the subject.

34. The method of claim 31, wherein the step of administering said therapeutic agent is performed by inhalation.

35. The method of claim 31, wherein the step of administering said therapeutic agent is performed by intranasal administration.

36. The method of claim 31, wherein said second moiety has said anti-allergic effect by at least significantly reducing degranulation of said mast cells.

37. The method of claim 36, wherein said second moiety is selected from the group consisting of a peptide, a polypeptide, a protein, a nucleic acid, a carbohydrate, a lipid, and a glycolipid.

38. The method of claim 37, wherein said second moiety is a peptide.

39. The method of claim 38, wherein said first moiety is a peptide.

40. The method of claim 39, wherein said linker is a covalent bond.

41. The method of claim 40, wherein said covalent bond is a peptide bond.

42. The method of claim 41, wherein said second moiety is a peptide taken from the C terminal sequence of Gαi₃.

43. The method of claim 42, wherein said molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.

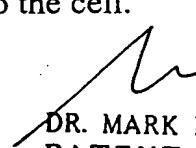
44. The method of claim 41, wherein said second moiety is a peptide taken from the C terminal sequence of Gαt.

45. The method of claim 44, wherein said molecule is a peptide having an amino acid sequence AAVALLPAVLLALLAPKENLKDCGLF.

46. The method of claim 40, wherein said therapeutic agent further comprises a second molecule, said second molecule being a peptide having an amino acid sequence AAVALLPAVLLALLAPKNNLKECGLY.

47. A method for promoting importation of a molecule into a cell of a subject *in vivo*, the method comprising the steps of:

- (a) attaching a leader sequence to the molecule, said leader sequence being a peptide having an amino acid sequence AAVALLPAVLLALLAP, to form a complex;
- (b) administering said complex to the subject; and
- (c) importing said complex into the cell through said leader sequence, such that the molecule is imported into the cell.



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References

Aridor M., Traub L.M. and Sagi-Eisenberg R. (1990). Exocytosis in mast cells by basic secretagogues: Evidence for direct activation of GTP-binding proteins. *J. Cell Biol.* 111:909-917.

Aridor M. and Sagi-Eisenberg R. (1990). Neomycin is a potent secretagogue of mast cells that directly activates a GTP-binding protein involved in. *J. Cell Biol.* 111:2885-2891.

Aridor M., Rajmilevich G., Beaven M.A. and Sagi-Eisenberg R. (1993). Activation of exocytosis by the heterotrimeric G protein G_{i3}. *Science* 262:1569-1572.

Bienzenstock J., Tomioka M., Stead R., Ernst P., Jordana M., Gauldie J., Dolovich J. and Denburg J. (1987). Mast cell involvement in various inflammatory processes. *Am. Rev. Respir. Dis.* 135:S5-S8.

Chahdi A., Mousli M. and Landry Y. (1998). Substance P-related inhibitors of mast cell exocytosis act on G-proteins or on the cell surface. *Eur. J. Pharmacol.* 341,329-335.

Columbo M., Horowitz E.M., Kagey-Sobotka A. and Lichtenstein L.M. (1996). Substance P activates the release of histamine from human skin mast cells through a pertussis toxin-sensitive and protein kinase C-dependent mechanism. *Clin. Immunol. Immunop.* 81,68-73.

Devillier P., Regoli D., Asseraf A., Descours B., Marsac J. and Renoux M. (1986). Histamine release and local responses of rat and human skin to substance P and other mammalian tachykinins. *Pharmacology* 32:340-347.

Emadi-Khiav B., Mousli M., Bronner C. and Landry Y. (1995). Human and rat cutaneous mast cells: involvement of a G protein in the response to peptidergic stimuli. *Eur. J. Pharmacol.* 272,97-102.

Ennis M., Pearce F.L. and Weston P.M. (1980). Some studies on the release of histamine from mast cells stimulated with polylysine. *Br. J. Pharmacol.* 70:329-334.

Foreman J.C. (1987a). Neuropeptides and the pathogenesis of allergy. *Allergy* 42:1-11.

Foreman J.C. (1987b). Substance P and calcitonin gene-related peptide: Effect on mast cells and in human skin. *Int. Archs. Allergy appl. Immun.* 82:366-371.

Goldberg A., Korzets Z., Bernheim J. And Mekori Y.A. (1991). Cutaneous responses to histamine, compound 48/80 and codeine in patients with chronic renal failure. *Annals Allergy* 67 :525-528.

Gomperts B.D., Churcher Y., Koffer A., Lillie T.H.W., Tatham P.E.R. and Whalley T. D (1991). Intracellular mechanisms regulating exocytotic secretion in mast cells. *Int. Arch. Allergy Appl. Immunol.* 94:38-46.

Hawiger J. 1997. Cellular import of functional peptides to block intracellular signaling. *Curr. Opin. Immunol.* 9: 189-194.

Kivity S., Sneh E., Greif J., Topilsky M. and Mekori Y.A. (1988). The effect of food and exercise on the skin response to compound 48/80 in patients with food-associated exercise-induced urticaria-angioedema. *J. Allergy Clin. Immunol.* 81:1155-1158.

Lichtenstein L.M (1993). Allergy and the immune system. *Scientific American* 269: 116-124.

Lin Y.Z., Yau S.Y., Veach R.A., Torgerson T.R and Hawiger J. 1995. Inhibition of nuclear translocation of transcription factor NF- κ B by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. *J.Biol.Chem.* 270:14255-14258.

Mousli M., Hugli T.E. Landry Y. and Bronner C. (1994). Peptidergic pathway in human skin and rat peritoneal mast cell activation. *Immunopharmacol.* 27:1-11.

Pearce F.L., Kassassinoff T.A., Liu W.L. (1989). Characteristics of histamine secretion induced by neuropeptides: Implications for the relevance of peptide-mast cell interactions in allergy and inflammation. *Int. Arch. Allergy Appl. Immunol.* 88:129-131.

Prochiantz A. 1996. Getting hydrophilic compounds into cells: lessons from homeopeptides. *Curr. Opin. Neurobiol.* 6:629-634.

Sagi-Eisenberg R. (1993). Signal-transmission pathways in mast cell exocytosis. In: *The Handbook of Immunopharmacology*. Academic Press, UK. pp. 71-88 (1993).

Sussman G.L., Harvey R.P. and Schochet A.L (1982). Evaluation of skin test response using two techniques of measurement. *Ann. Allergy* 48:75-77.

Theoharides T.C. (1996). The mast cell: a neuroimmunoendocrine master player. *Int. J. Tissue React.* 18:1-21.

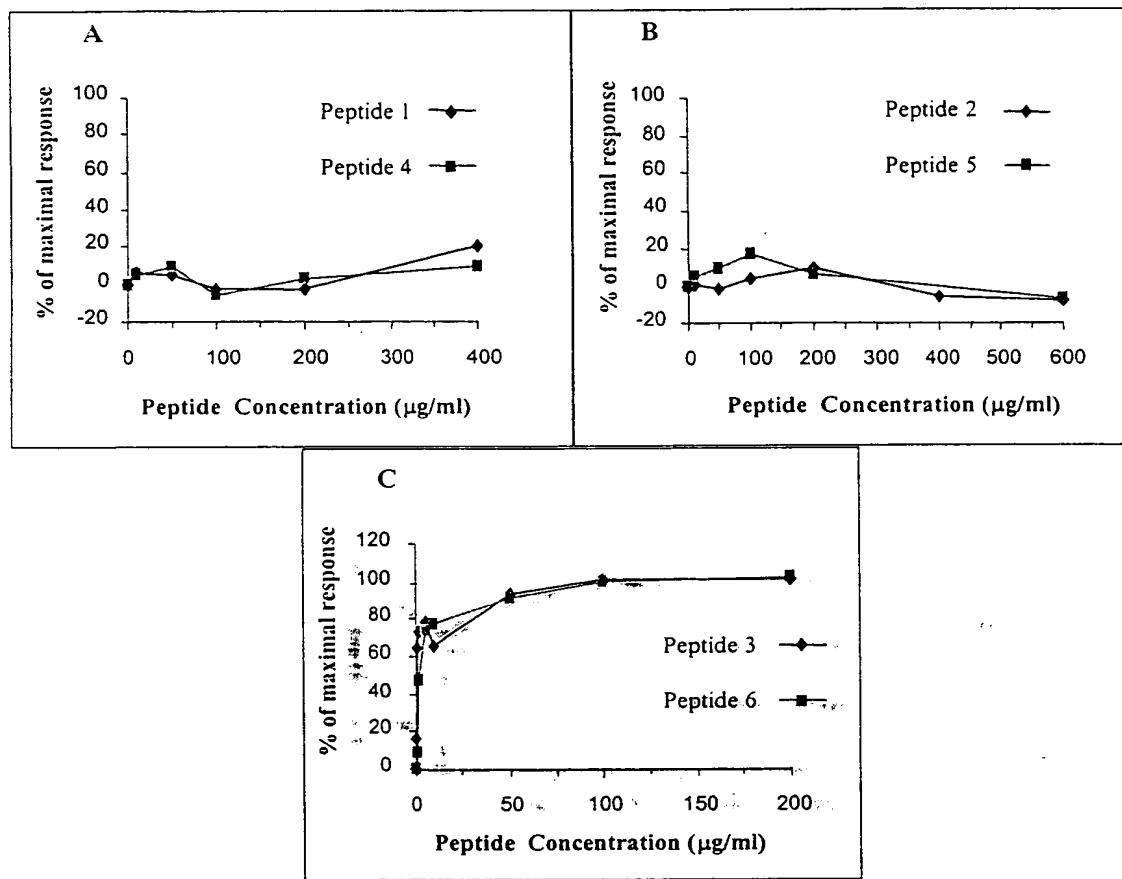


Fig 1: Effect of different peptides on histamine secretion. Purified mast cells (10^5 cells/0.5ml) were incubated with buffer or with different concentrations of the indicated peptide for 2 h. at 37°C . At the end of the incubation period the cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as percentage of the maximal response induced by 5 μg/ml compound 48/80 taken as the reference for allergen induced secretion, which corresponds to 60% of the total cellular histamine content.

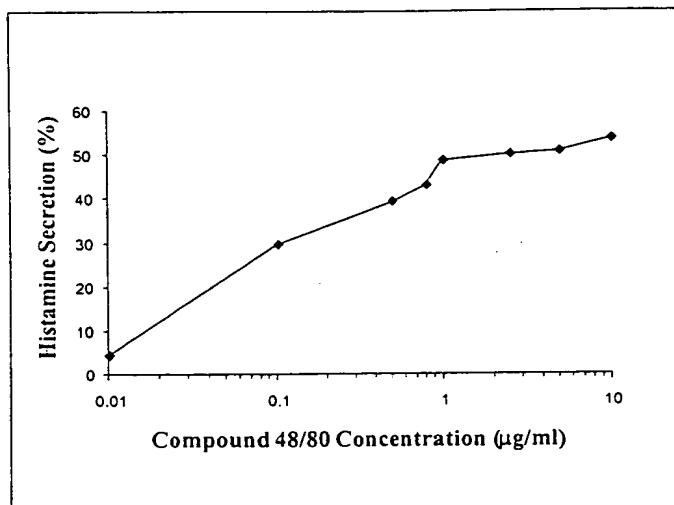


Fig 2: Dose response effect of compound 48/80 induced histamine release from intact mast cells. Purified mast cells (10^5 cells/0.5ml) were incubated with buffer for 2 h. at 37°C. Following the 2h. incubation histamine secretion was stimulated by the indicated concentrations of compound 48/80. At the end of a 20 min incubation period the reaction was terminated by placing the tubes in ice. The cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as the net secretion, that is % of the total cellular histamine following subtraction of the spontaneously released histamine. A representative experiment is demonstrated.

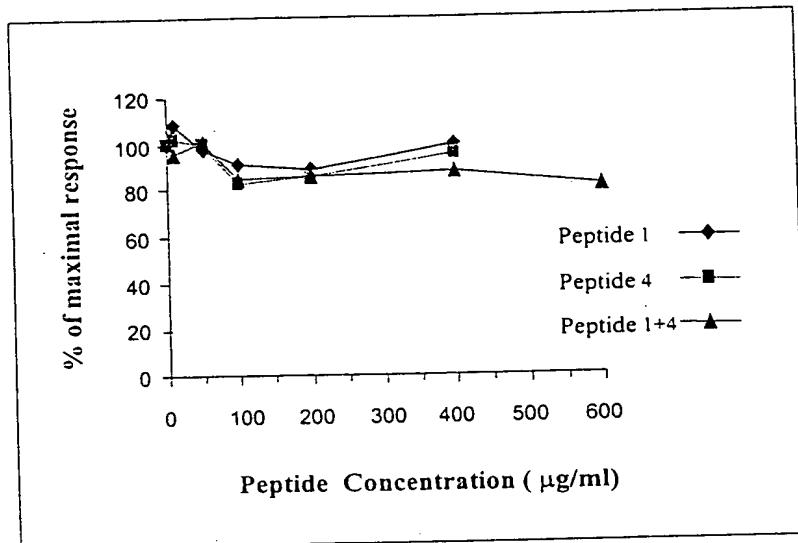


Fig 3: Blocking effect of peptides 1 and 4 on histamine secretion. Purified mast cells (10^5 cells/0.5ml) were incubated with buffer or with different concentrations of the indicated peptide for 2 h. at 37°C . Following the 2h. incubation histamine secretion was stimulated by 5 $\mu\text{g}/\text{ml}$ of compound 48/80. At the end of a 20 min incubation period the reaction was terminated by placing the tubes in ice. The cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as percentage of the maximal response, which corresponds to 60% of the total cellular histamine content.

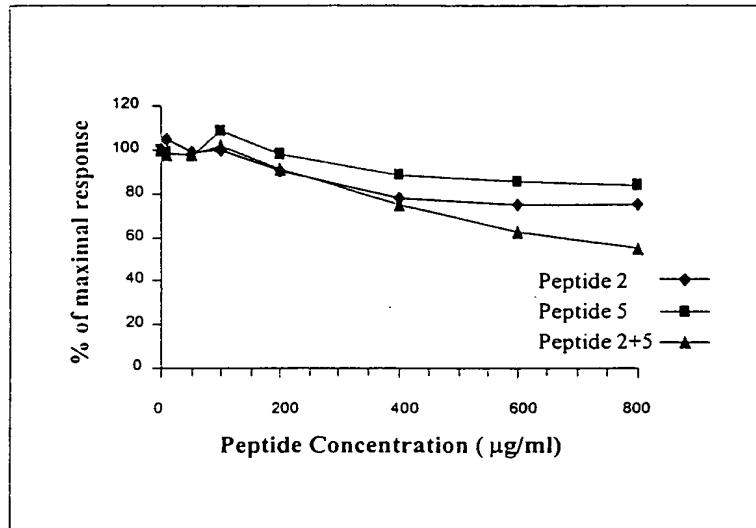


Fig 4: Blocking effect of peptides 2 and 5 on histamine secretion. Purified mast cells (10^5 cells/0.5ml) were incubated with buffer or with different concentrations of the indicated peptide for 2 h. at 37°C . Following the 2h. incubation histamine secretion was stimulated by 5 $\mu\text{g}/\text{ml}$ of compound 48/80. At the end of a 20 min incubation period the reaction was terminated by placing the tubes in ice. The cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as percentage of the maximal response, which corresponds to 60% of the total cellular histamine content.

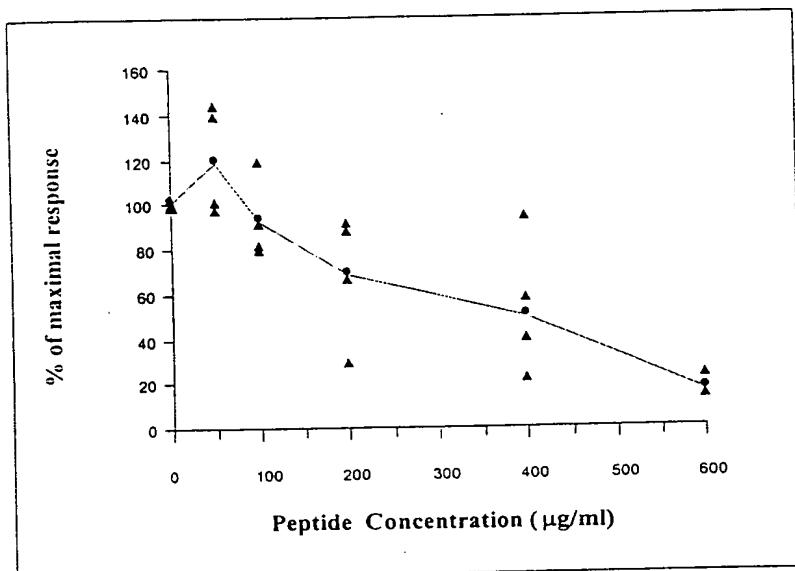


Fig 5: Blocking effect of peptide 2 on histamine secretion. Purified mast cells (10^5 cells/0.5ml) were incubated with buffer or with different concentrations of the indicated peptide for 2 h. at 37°C . Following the 2h. incubation histamine secretion was stimulated by 0.1 $\mu\text{g}/\text{ml}$ of compound 48/80. At the end of a 20 min incubation period the reaction was terminated by placing the tubes in ice. The cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as percentage of the maximal response, which corresponds to 20% of the total cellular histamine content. Each experiment is indicated by data points \blacktriangle which represent the average of duplicate measurements. The average histamine secretion for each peptide concentration is indicated \bullet .

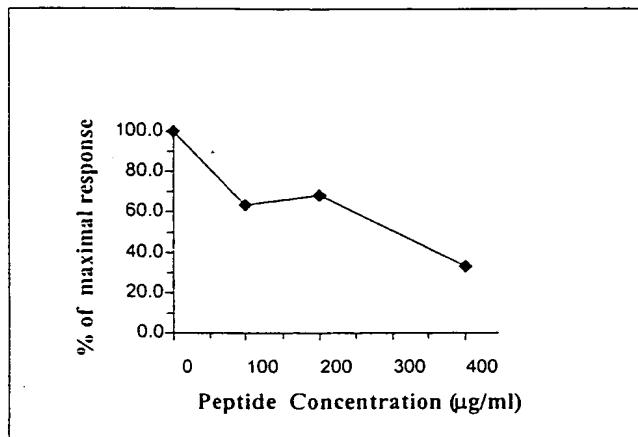


Fig 6: Blocking effect of peptide 2 on substance P induced histamine secretion.

Purified mast cells (10^5 cells/0.5ml) were incubated with buffer or with different concentrations of the indicated peptide for 2 h. at 37°C . Following the 2h. incubation histamine secretion was stimulated by 50 μM of substance P. At the end of a 20 min incubation period the reaction was terminated by placing the tubes in ice. The cells were sedimented and the supernatants were collected. Histamine release was determined and is presented as percentage of the maximal response, which corresponds to 22% of the total cellular histamine content. X-axis shows peptide concentration (micrograms per milliliter), and y-axis shows % of maximal response.

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